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BIOSENSING TECHNIQS FOR HUMAN DETECTION,

II. THE FROG SKIN TRANSDUCER:

A CONTINUOUS FLOW SYSTEM FOR MAKING CRITICAL MEASUREMENTS

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Title

BIOSENSING TECHNIQS FOR HUMAN DETECTION —

II. THE FROG SKIN TRANSDUCER:

A CONTINUOUS FLOW SYSTEM FOR MAKING CRITICAL MEASUREMENTS

BY

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ABSTRACT

Earlier results in which transmembrane potential changes were elicited across frog belly skin by a number of compounds were duplicated. Four additional compounds were tested, including dimethyl sulfoxide. Dimethyl sulfoxide has no apparent adjuvant action when tested with urea. Measurements were attempted using low concentrations of test compounds by an expanded scale technique. The static 2-chamber cell is not suitable for this type of measurement. A continuous flow cell was assembled and tested. This system should permit critical measurement of effects of low concentrations of test substances and it also provides a point of departure for design studies leading to development of a practicable field detection instrument.

FOREWORD

This report describes experiments carried out during the period November, 1964 through March, 1965. The work was conducted under U.S. Army Limited War Laboratory Task 02-B-64, Instrumented Biosensors. The senior author is President of the Biosearch Company, Newton, Massachusetts.

I. INTRODUCTION

The need for an instrument for sensitive and immediate detection of the presence of concealed humans has led to consideration of the biosensing approach described here and in an earlier report.¹

Biosensing involves the use in real time of living material as the primary sensor in an instrument system, and represents a plausible alternative technique to the use of inanimate sensors utilizing chemical principles. The frog skin sensor currently under study in this laboratory typifies the use of "barrier tissue", whose properties may be altered by exposure to chemical substances, with consequent changes in transmembrane electrical potential.

In the work reported here, the following objectives were achieved:

1. Check data on substances previously studied² were obtained to determine if the earlier results are reproducible.
2. Data on new selected substances were obtained.
3. The effect of dimethyl sulfoxide (DMSO) as an adjuvant was examined.
4. Measurements were made with lower concentrations of test compounds, and technical factors limiting sensitivity were explored.
5. Continuous flow measurement equipment and techniques were devised, making possible critical measurements of low concentrations of test compounds.

II. METHOD AND APPARATUS

A. Double chamber static cell.

The double chamber polyethylene cell described earlier was used to record additional data in the present series of experiments.¹ In this assembly, changes in electrical potential across a piece of frog belly skin mounted between two (2) 30cc polyethylene chambers are monitored via Beckman Ag: AgCl electrodes connected to a Keithley electrometer amplifier and a Varian recorder.

Ten compounds shown in Table 1 were tested for their effect on the transmembrane potential of frog belly skin. Four compounds were used for the first time; these were decanoic acid, squalene, putrescine and dimethyl sulfoxide; responses

obtained with six of the compounds were reported previously.¹ A total of 72 measurements was made in the present series and the substances listed were applied in the concentrations shown in the table. All of these compounds except DMSO and putrescine are known to be present in human effluents.²

TABLE I

Compounds tested for effect on transmembrane potential of frog belly skin

| Substances | Concentration, ppm |
|----------------------------|-------------------------|
| Indole | (4); 10; 100; 250; 500 |
| Lysine | 20; 50; 200; 1000; 2000 |
| Hexanoic Acid | (2); (4); (6.5); 20 |
| Urea | 400; 50,000; 100,000 |
| Skatole | 10; 100 |
| Taurine | 1000 |
| *Decanoic Acid | 100 |
| *Squalene | 200 |
| *Putrescine | 1000 |
| *Dimethyl Sulfoxide (DMSO) | 5000; 10,000 |

For testing each substance was dissolved or suspended in frog Ringer solution, the pH of which was then adjusted to 7.0. Materials of low solubility in water (squalene, hexanoic acid, decanoic acid) were emulsified in Ringer solution with a Branson sonifier and used immediately.

A protocol for a typical experiment is shown in Figure 1. After an initial equilibration period, in which a reference potential is established, one chamber is

1. UREA 100 mg%. $A_U::A_{fr}::B_U::B_{fr}::*$
2. UREA 1 g%. $B_U::B_{fr}::A_U::A_{fr}$
3. LYSINE 5 mg%. $B_{fr}::B_l::B_{fr}::A_l::A_{fr}::$
4. LYSINE 100 mg%. $A_l::A_{fr}::B_{fr}::B_l::B_{fr}::$
5. TAURINE 100 mg%. $A_{fr}::A_t::A_{fr}::B_t::B_{fr}::$
6. PITRESSIN 2U%. $B_p::B_{fr}::A_{fr}::A_p::A_{fr}::$
7. PUTRESCINE 1pp mg%. $B_{fr}::B_{pu}::B_{fr}::A_{fr}::A_{pu}::A_{fr}::$
8. INDOLE 1 mg%. $A_i::A_{fr}::B_{fr}::B_i::B_{fr}::$
9. INDOLE 25 mg%. $A_i::A_{fr}::B_i::B_{fr}::A_{fr}::$
10. INDOLE 50 mg%. $A_i::A_{fr}::B_i::B_{fr}::$
11. SKATOLE 1 mg%. $B_{sk}::B_{fr}::A_{sk}::A_{fr}::$
12. SKATOLE 10 mg%. $B_{sk}::B_{fr}::A_{sk}::A_{fr}::$
13. HEXANOIC ACID 2 mg%. $A_h::A_{fr}::B_h::B_{fr}::$
14. HEXANOIC ACID 20 mg%. $A_h::A_{fr}::B_h::B_{fr}::$
15. SQUALENE 0.02 cc%. $B_{sq}::B_{fr}::A_{sq}::A_{fr}::$
16. DECANOIC ACID 10 mg%. $B_d::B_{fr}::A_d::A_{fr}::$
17. UREA 1 g%. $A_U::A_{fr}::B_U::B_{fr}::$

Figure 1. Typical experimental protocol.

* A_{fr} = empty, then fill with Ringers, side A (inside of skin)

B_U = empty, then fill with urea solution, side B (outside)

:: = after measurement, follow this procedure by next letter item

emptied, then filled with the test solution. The potential change across the skin at two minutes is noted, and the maximum change is also recorded. The test chamber is then emptied and filled with fresh plain Ringer solution. Return of the transmembrane potential to the original value or to a new reference level is awaited before a new substance is applied.

In another short series, substances known to act with greater effect on one side of frog skin than the other were added to both chambers of the cell simultaneously, to observe differential effects.

In one experiment DMSO, because of its reputation as a tissue permeant, was added to observe its effects as an adjuvant or in enhancing the effect of a test substance. In one run, DMSO exposure and flush preceded addition of the test substance. In another run, DMSO was added simultaneously with the test substance (urea).

Observations were made on the capability of the double chamber system to yield high sensitivity data. The gain of both the amplifier and the recorder was increased 100 times and appropriate zero adjustments were made to bring a one (1) millivolt full scale indication onto the chart. Control records of the drift that is normally observed with this type of apparatus were obtained. Test solutions of lysine, indole, DMSO and hexanoic acid, were added dropwise.

B. Flow cell system.

A continuous flow apparatus was designed and assembled in which a fluid circulating system for each half-cell was provided. The potential advantages of such a system over the original static cell assembly are several. Thus, in a circulating system, repeated exposures of a piece of skin to several passes of the same mixed fluid are possible. Such a system also permits the introduction and removal of mixes of desired composition with minimal disturbance from extraneous variables. Any desired condition can be readily stabilized, for example fluid volume flow rate, temperature, chemical composition of the medium, electrical and mechanical factors. It is feasible to employ smaller pieces of frog skin (under 1 cm²) than in the static assembly, and to reduce the chamber volume on each side of the skin to less than 20cc. Additional advantages of the circulating system were ease of fabrication, assembly and replacement of parts.

A view of a flow cell assembly is shown in Figure 2. Temperature control was not provided in the system shown. It is a simple matter to include a thermostatted heating device in the assembly, as was done later. Figure 3 shows a black

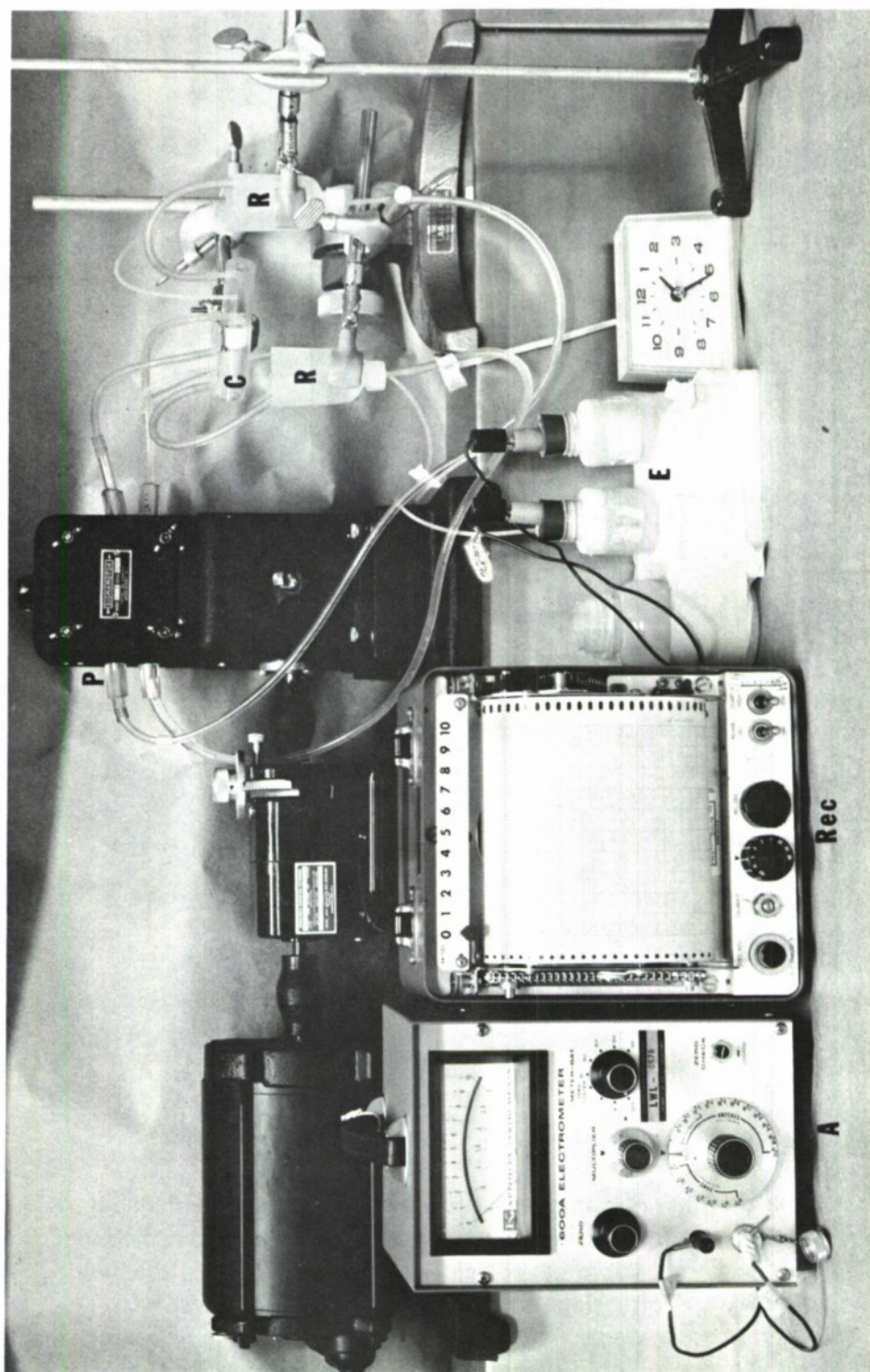


Figure 2. Continuous flow set up. A, Electrometer - amplifier; C, double chamber cell; E, electrode assembly; P, pump; R, fluid reservoir; Rec, recorder.

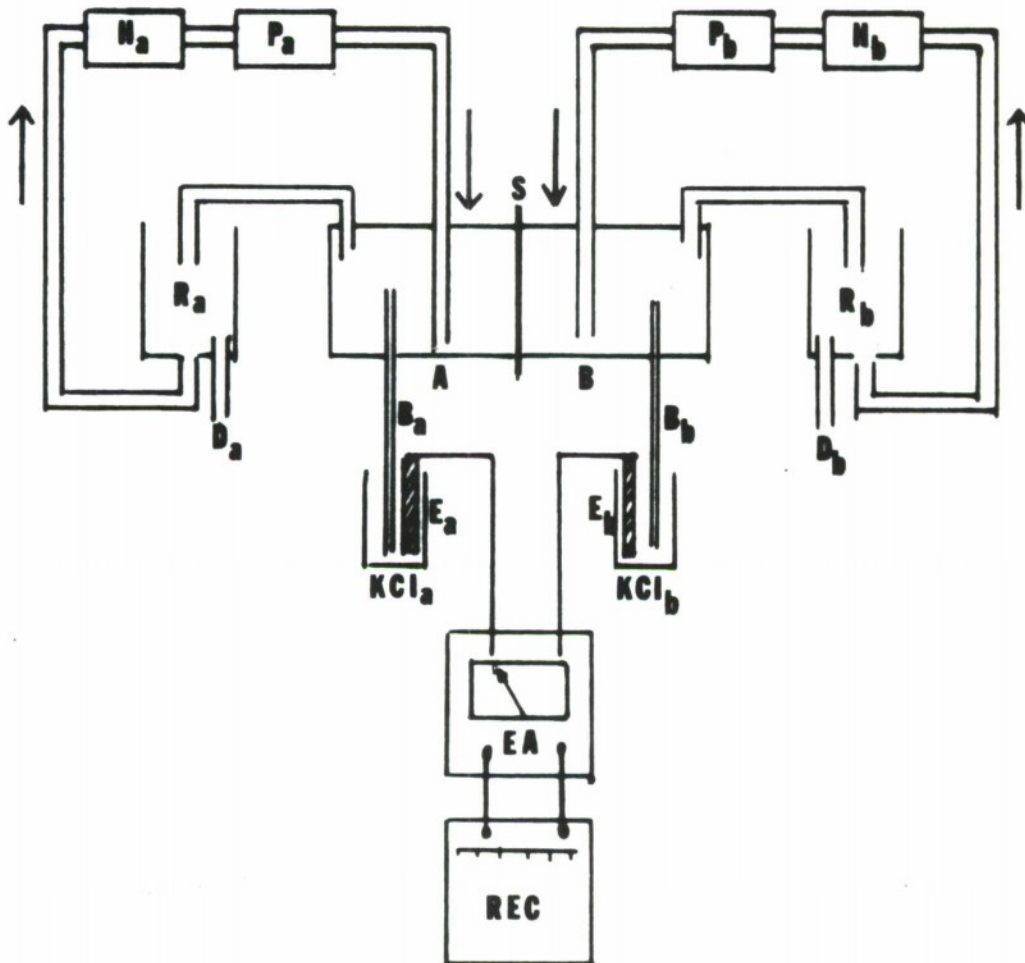


Figure 3. Block diagram of continuous flow system. A, B, 2-chambered test cell; S, frog belly skin; H_a, b , helical electric resistance heaters; P_a, b , valveless peristaltic pumps; R_a, b , fluid reservoirs; B_a, b , salt bridges; E_a, b , Ag: AgCl electrodes; KCl_a, b , KCl electrode vessels; EA, electrometer amplifier; REC, recorder. Arrows indicate direction of flow in the two sides of system.

diagram of the flow-through system which includes a helical resistance heater to permit close control of fluid temperature.

The flow through cell, a photograph of which is shown in Figure 4, is made of two (2) small pill vials, each cut to a volume of about 10cc. Three plastic tubes are set into each vial. One tube is for fluid inflow, one for outflow and the third for an electrode. A piece of frog belly skin is placed over the open end of one vial where it is secured by a snap-on vial top with the center cut out, forming simply a retaining ring. The second vial is then joined to the skin-bearing vial with some pressure by a short piece of polyethylene tubing.

In typical operation of the continuous flow system, liquid flow rates of about 1cc/sec are maintained. Fluid is pumped through each side of the cell under a pressure of about 3 psi, then into each of 2 reservoirs in which it is at atmospheric pressure. The fluid is reheated to the required temperature between the reservoirs and the two (2) channel, valve-less peristaltic pump (Sigmamotor). Test substances can be added as desired to the circulating fluid in the reservoirs. Flushing and rinsing the system are easily accomplished with minimal disturbance of the skin.

III. RESULTS

Transmembrane potential changes across frog belly skin were observed in the static cell assembly used previously with each of the substances listed in Table 1 when applied in the concentrations shown. Mean values and variability of potential changes seen with the various concentrations of indole, lysine, hexanoic acid, urea, skatole and taurine agreed closely with those reported earlier.¹ Confirmation of a phenomenon recorded in the previous experiments was also obtained. The curves shown in Figure 5 were constructed from the new data for lysine and indole. These show again that at low concentrations of these compounds, there is a reversal of the direction of potential change from negative to positive.

The present series of experiments also confirmed the observation made earlier that there is a high degree of reversibility and reproducibility for a given piece of frog skin with repeated exposures to a given substance.

In experiments in which a substance, known to produce a greater effect on one side of the skin than on the other was added to both chambers of the test cell simultaneously, a net change of potential in one direction was observed. The mean value from a limited number of tests of this sort correspond well with the mean value of pooled separate inside and outside measurements with the same substance.

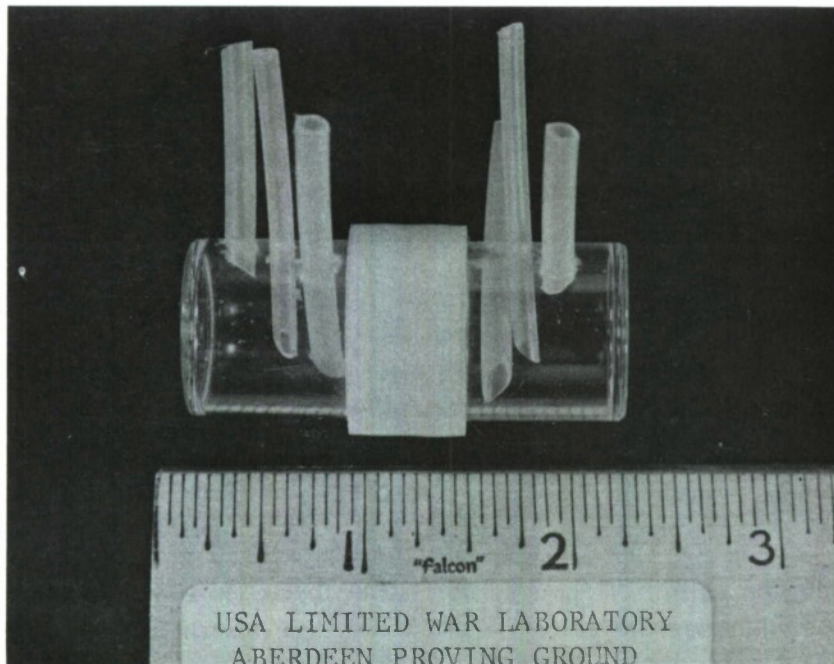


Figure 4. Two-chambered cell for use in continuous flow system.

Under the conditions of these experiments, it was found, as before, that frog skin is usable for continuous periods as long as 8 hours.

No enhancing or adjuvant effect of dimethyl sulfoxide was observed when it was mixed in varying proportions with urea. Dimethyl sulfoxide alone elicits change in the transmembrane potential when applied in the concentrations shown in Table 1.

When amplifier-recorder sensitivities are increased 100-fold, and appropriate zero adjustments are made to give a signal at one (1) mv full scale with the static 2-chambered cell, background noise on the order of 5 to 20 microvolts and continuous drift occur as shown by the typical control record reproduced in Figure 6A. In spite of the noise level and the drift, small changes on this baseline could be observed in response to the addition of test substances to the cell. Representative tracings are shown in Figures 6B, 6C and 7A.

A limited number of test runs with the continuous flow system was completed. Preliminary trials were performed using 1 per cent urea in frog Ringer solution as the test substance. A representative recording showing the effect of urea addition is reproduced in Figure 7B. Most of the "noise" in this recording was due to the pump, and was later largely eliminated.

IV. DISCUSSION

The expanded scale technique for increasing measurement sensitivity is of limited value with the static assembly in which fluid changes are accomplished by draining and refilling the cell chambers. These manipulations create a variety of mechanical, thermal, chemical and electrical disturbances. The continuous flow system, on the other hand, lends itself admirably to application of the expanded scale technique. Disturbance of the system occasioned by the introduction and withdrawal of test substances is minimized. At the present time it is reasonable to predict that systematic exploitation of continuous flow procedures may well extend the detection capability of the frog skin system into the parts per billion range with selected compounds. The variability of response hitherto observed among different skin samples and between different trials with static systems was undoubtedly due in large part to the manipulations necessary to carry out the test protocols.

Guidelines for achieving further progress toward the ultimate goal of a feasible field instrument for detecting people can be indicated at this time. These include:

1. systematic exploitation of the potential capabilities of the continuous flow system;

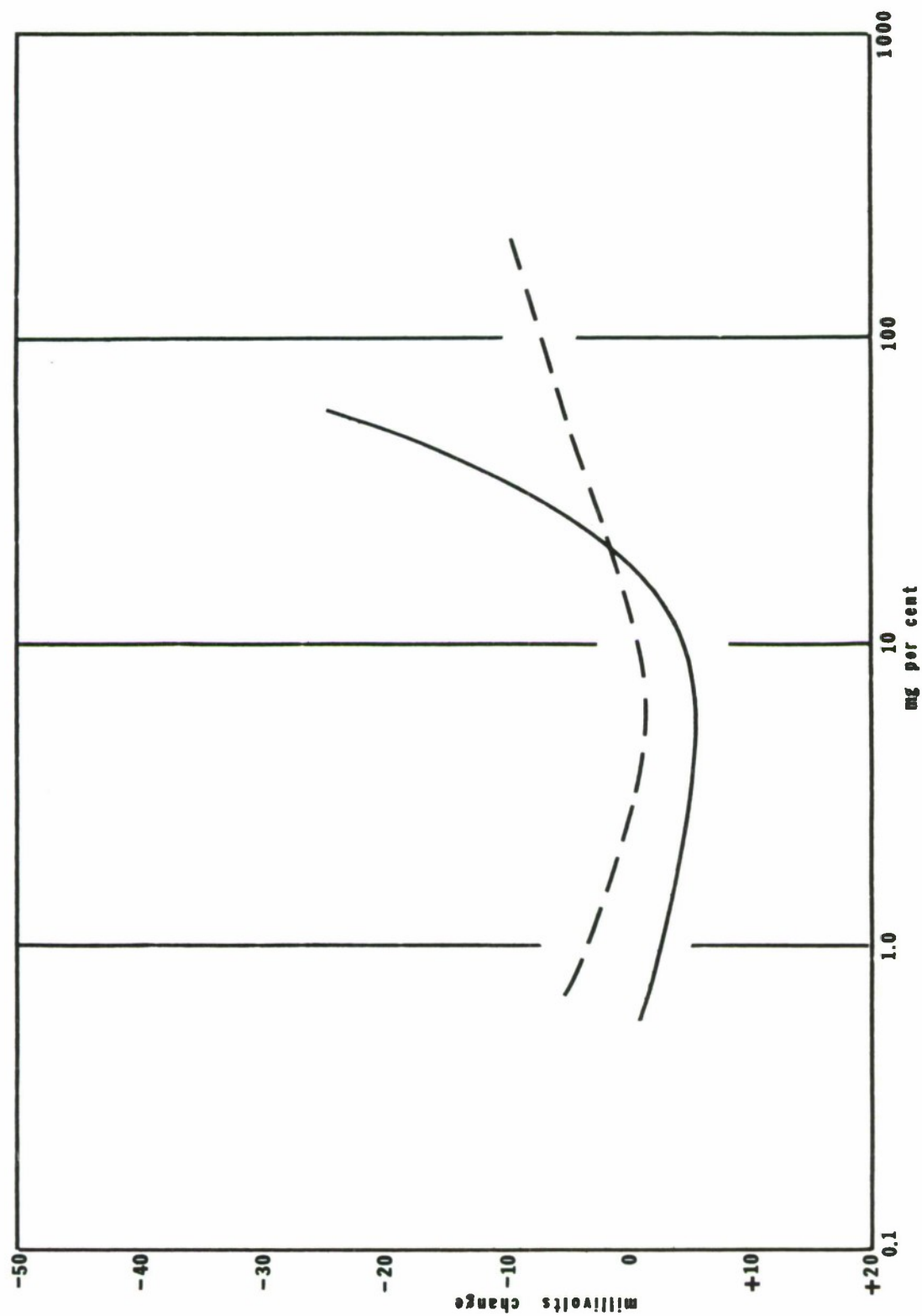


Figure 5. Transmembrane potential changes across frog belly skin elicited by solutions of indole and lysine. Solid line is indole; broken line is lysine.

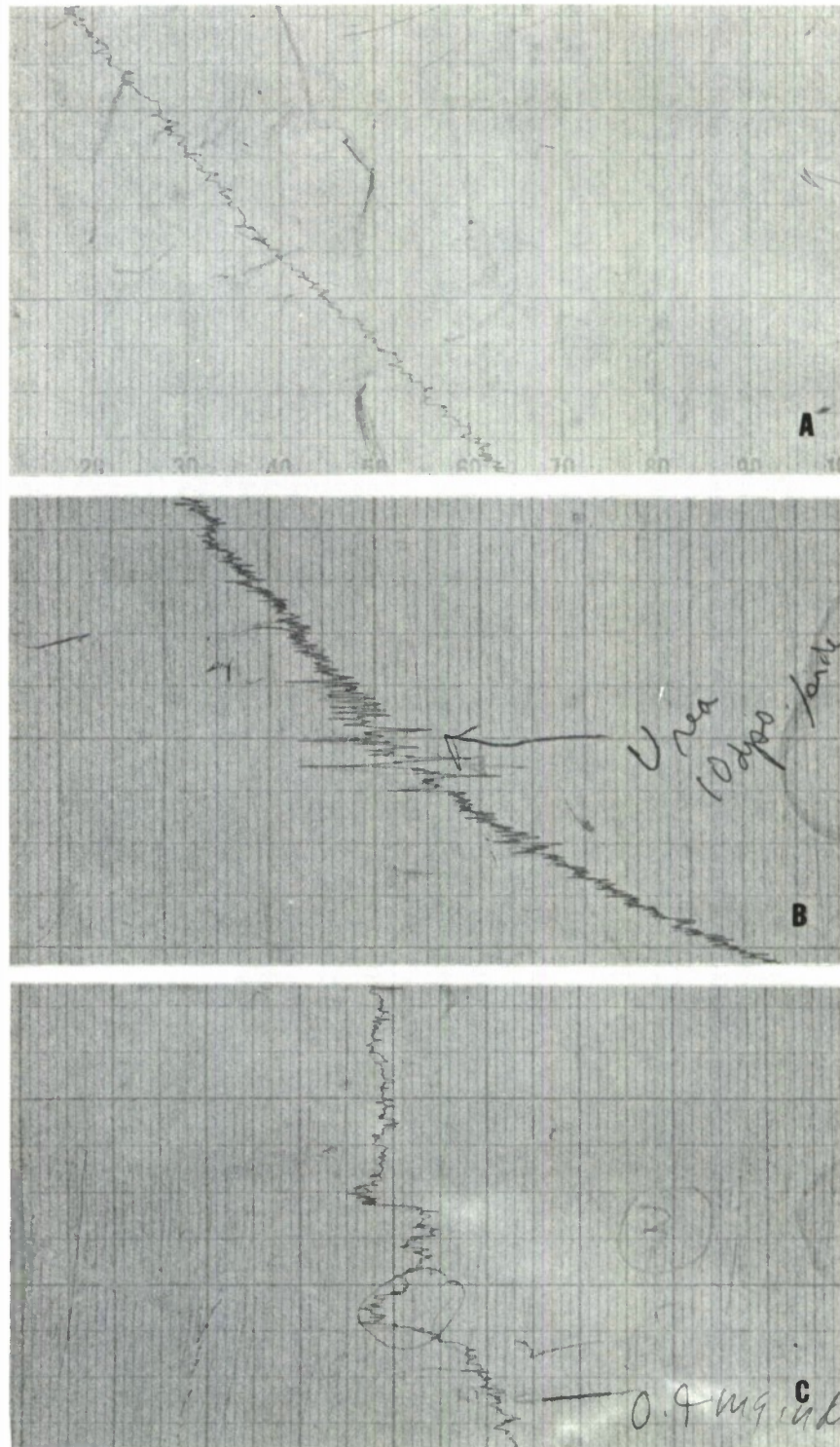


Figure 6. Expanded scale records using static 2-chambered cell system. A. Control. B. Urea, 1 per cent. C. Indole, 0.4 mg per cent.

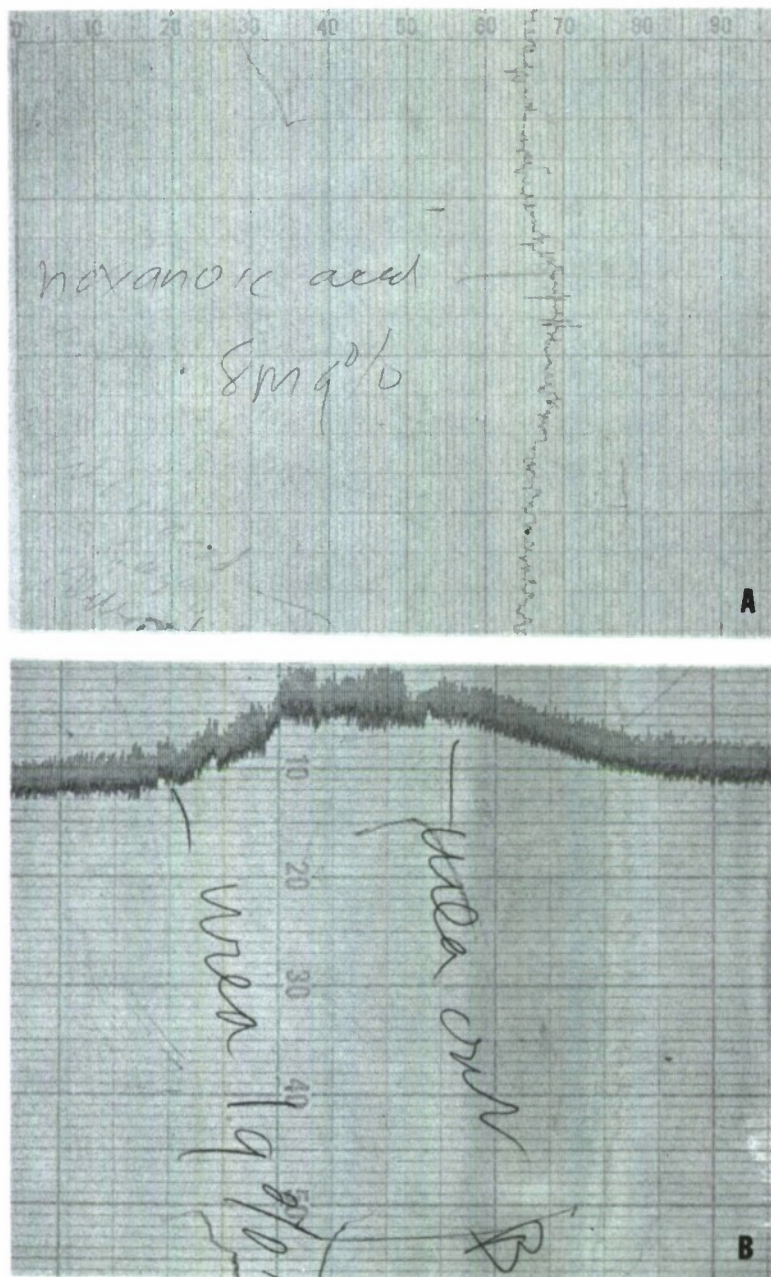


Figure 7. Expanded scale measurements.
 A. Hexanoic acid, 0.8 mg per cent, static system. B. Urea, 1 per cent, continuous flow system.

2. design studies leading to a prototype field instrument.

The continuous flow system described in this report can be used as a point of departure for design of a field instrument. Certain requirements for such an instrument can be stated now, and include (a) a front end air sampler to concentrate intake air into a small volume of liquid, (b) overall miniaturization, (c) use of disposable, easily assembled modular components of inert plastic. A conventional, hand-carried Radiac meter of the ionization type, with its electronic amplifier, circuitry and indicator, can possibly be adapted for use as part of such a field assembly.

V. SUMMARY

1. Further experiments with a static 2-chamber cell assembly gave results that were in essential agreement with earlier test data.

2. Four new compounds were tested for their effect on the transmembrane potential of frog belly skin. These were decanoic acid, squalene, putrescine and dimethyl sulfoxide.

3. Dimethyl sulfoxide in relatively high concentration causes a detectable change in transmembrane potential. It does not, however, exhibit a demonstrable enhancing or adjuvant effect when combined with urea.

4. An expanded scale technique for increasing the sensitivity of measurement was explored. Background noise and drift are correspondingly enhanced. The static system is not suited to application of this technique.

5. A continuous flow system was assembled and tested. Preliminary runs were made using 1 per cent urea as a test substance. This system seems well-suited for exploitation of the expanded scale technique to make critical measurements of the effects of low concentrations of test substances.

6. The continuous flow assembly provides a point of departure for design studies leading to development of a practicable field detection instrument.

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